ECOFRIENDLY SYNTHESIS OF CERIUM NANOPARTICLES WITH AMLA FRUIT (EMBILICA OFFICINALIS) AND ITS ANTICANCER ACTIVITY

Rohini. Pol* and Mallikarjun S.Yadawe

Department of Botany* S.V.M College Ilkal and Chemistry S.B. Arts and K.C.P. Science College Vijayapur, Karnataka, India.

Abstract:-The recent development and implementation of new technologies have led to a new trend, the nano-revolution unfolding the role of plants in bio- and green synthesis of nanoparticles which seems to have drawn a quite unequivocal attention to the synthesis of stable nanoparticles. The synthesized nanoparticles were characterized by UV-Vis spectroscopy, FTIR, X-ray diffraction energy dispersive X-ray analysis and scanning electron microscopy. The results showed that the prepared CeNPs were nearly spherical in shape with sizes ranging from 1µm to -100.With the aim of making specific targeting of cerium nanoparticles as a drug for tumour cells and developing new anticancer agents, a novel nano-composite was developed. Cerium nanoparticles and their cancerous effects were evaluated against MDA-MB 231, a human breast cancer cell line.The results indicate that cerium nanoparticles could be a good candidate for chemotherapeutic drug.

Key words:-Amla, nanoparticles, synthesis, scanning electron microscopy, anticancer activity.

Introduction:-Nanotechnology includes various fields of science such as surface science, organic chemistry, molecular biology, semiconductor physics and micro fabrication [1, 2].Nanotechnology has the potential to create mainly new materials and devices with vast range of applications, such as in medicine, electronics, biomaterials and energy production etc. Nanotechnology is considered an emerging technology due to the possibility to advance well established products and to create new products with totally new characteristics and functions with enormous potential in a wide range of applications. In addition to various industrial uses, great innovations are foreseen in information and communication technology, in biology and biotechnology, in merology. However, to enhance various properties of nanomaterials to meet the increasing needs for different applications. It is needed to reduce the size and increase the active surface area of nonmaterial's. Decrease in the particle size enhancing conductivity, electrical, sensing and catalytic properties of nonmaterial [3, 4, 5]. Amla, being a rich source of vitamin C, is considered to be effective in slowing down the ageing process. Ageing is a cumulative result of damage to various cells and tissues, mainly by oxygen free radicals. Vitamin C is a scavenger of free radicals which breaks them down: it has an antioxidant synergism with vitamin E which prevents pre-oxidation of lipids. Amla is major ingradient of ancient ayurvedic preparation "Chyawanprash" which believed to prolong the ageing process and helps to keep young[6].The fruits of plants have been used in ayurveda as a rasayana [7].

In the present study, we have made an attempt to investigate the anticancer effect of cerium nanoparticles. The formed nanoparticles was studied by scanning electron microscopy, FTIR and PXRD method.

Materials and Methods

Synthesis of Cerium Nanoparticles

Amla fruits were purchased from the Vijayapur local market. The extraction sample was prepared by extracting the juice of the fruits, sieving it and storing it for the synthesis of CeNPs. Both fresh and refrigerated extract were used and they yielded similar results. An aqueous solution of 0.1M thorium nitrate was prepared. Amla extract was added to thorium nitrate at volumetric ratio of 1mL amla extract to 10mL ceric sulphate. A colour change was observed within approximately 5 minutes of the reaction.

SEM Analysis of Cerium Nanoparticles: Scanning Electron Microscope (SEM) analysis was done using (JEOL Model JSM - 6390LV) SEM machine. The films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

Fourier Transform Infrared: Dried powder of the CeNPs was subjected to analyze the presence of possible functional groups for resulting in formation of CeNPs using Fourier transform infrared (ATR schimadzu Japan) spectroscopy..

X-Ray Diffraction Analysis: To determine the nature and size of the synthesized CeNPs, X-ray diffraction (XRD) was performed using on an Xpert Pro MPD, which was operated at a voltage of 40 kV and current of 40mA with Cu-Kα radiation.

Anticancer activity (SRB ASSAY): The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

Experimental drugs were initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature.

After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100.

Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

[Ti/C] x 100 % [8, 9].

Results and Discussion

SEM with EDX Analysis of Cerium Nanoparticles: SEM of cerium nanoparticles is shown in fig.1. Surface and morphological characterization of cerium nanoparticles were carried out using scanning electron microscopy. Nanosized spherical shaped cerium particles obtained confirmed. The mean size of the particles varies from 1 to 100µm.The sphere like shaped of the particles with clumped distributions are visible through SEM analysis. The prepared sample is not agglomerated. The energy dispersive X-ray analysis of the cerium and oxygen elements which confirms the purity of the sample.

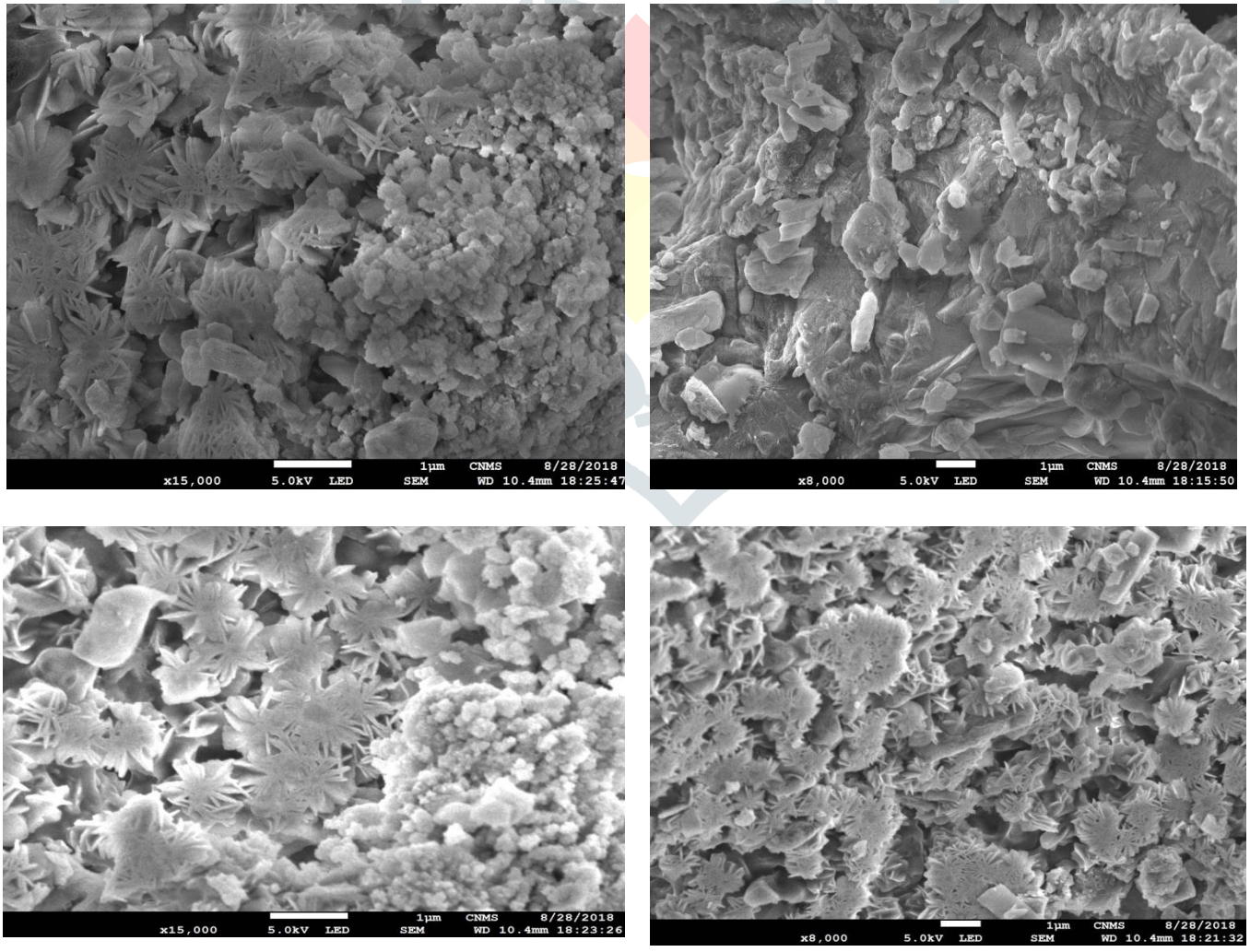


Fig.1 (a,b,c and d) SEM images of Cerium nanoparticles

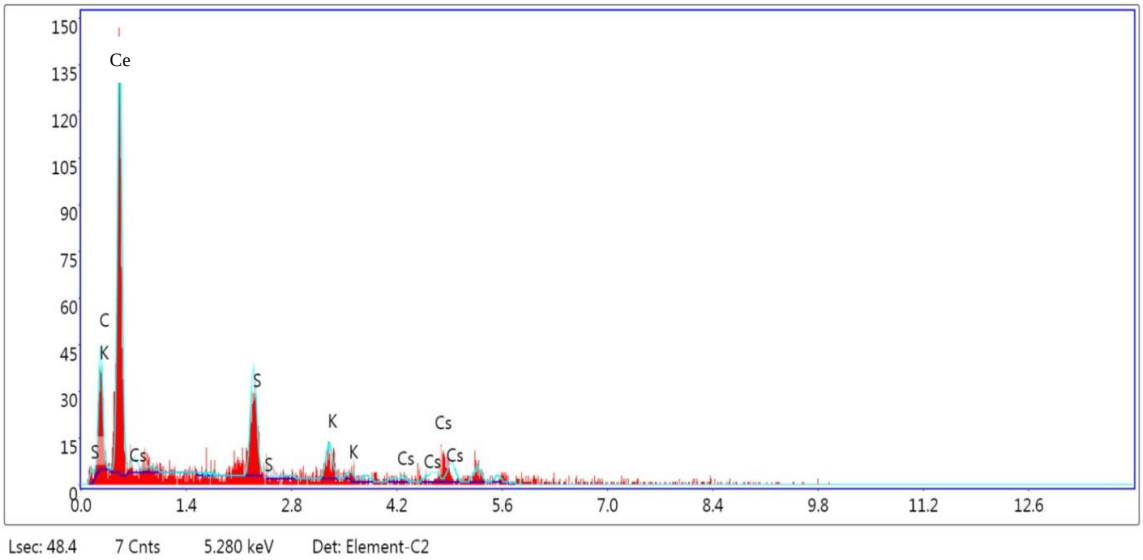


Fig.2. EDX of Cerium nanoparticles.

Fourier Transform Infrared Spectrum: The particles were capped with polyphenols present in amla biomolecules stabilized nanoparticles and capping of potent biomolecules was confirmed by FTIR. FTIR spectra revealed the strong bands at 3327, 1745, 1625 and 1050 cm^{-1}. Similarly band at 3380 cm^{-1} due to functional group in alcohols and phenolic compounds shifted to 3420cm^{-1} for cerium nanoparticles. For bands 1725 cm^{-1} and 1619 cm^{-1} in the curve shifted to 1745cm^{-1} (carbonyl groups) and the weaker band at 1058 cm^{-1} was shifted to 1025 cm^{-1} (C-O-C and C-OH vibrations of the proteins). In FTIR spectra the band shifted at 1720,1618,and 1058 cm^{-1}(Fig 0).In this paper the approach employed in the production of these materials is low cost and ecofriendly. The Amla fruit extract band 645 was shifted to 650 $cm^{-1}$and it was responsible for C-H stretch [8-10].

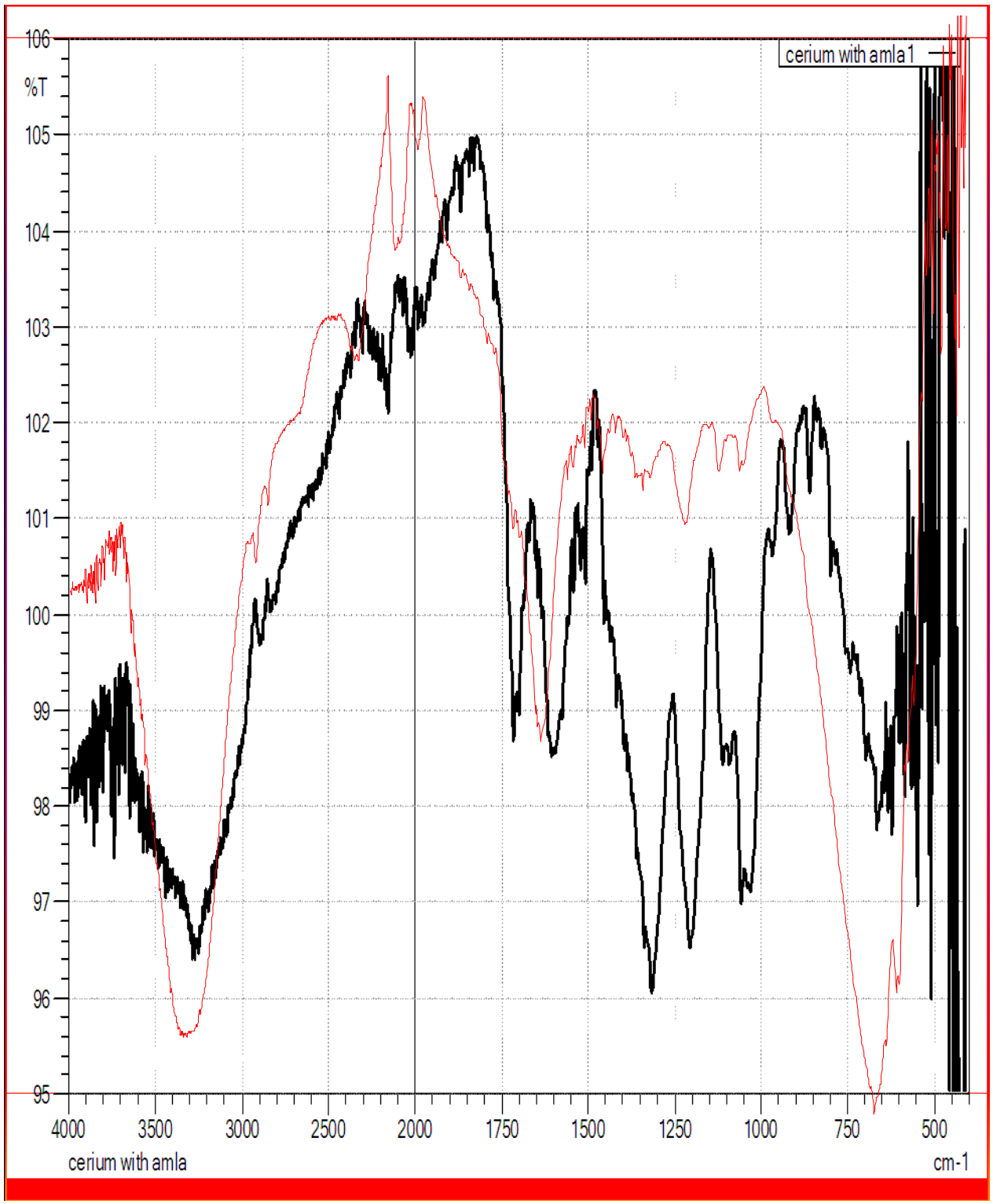


Fig.3. FTIR spectra of Amla(Red lines) and nanoparticles (Black lines)

X-Ray Diffraction Analysis: In the present study XRD spectrum with the standard confirmed that the cerium particles formed in our experiments were in the form of monoclinic (Primitive) system of ZrNPS (JCPDS Data NO.89-9066,) which can be confirmed from the peaks obtained at 2 theta angles. The Fig.4 shows peaks located at angles of (2 theta) of 17.43,24.06,28.21,31.45,34.15,35.29,38.84,40.76,44.79,50.61,54.03 correspond to (300,122,221,013,113,031,130,310,311,312,320) planes pf ZrNPs. Deby Scherrer formula[11] was used to

determine the averge crystalline size of te particles. The avarage crystalline size was estimated as nm for CeNPs. The average crystalline size was estimated at 16.13 nm for CeNPs.

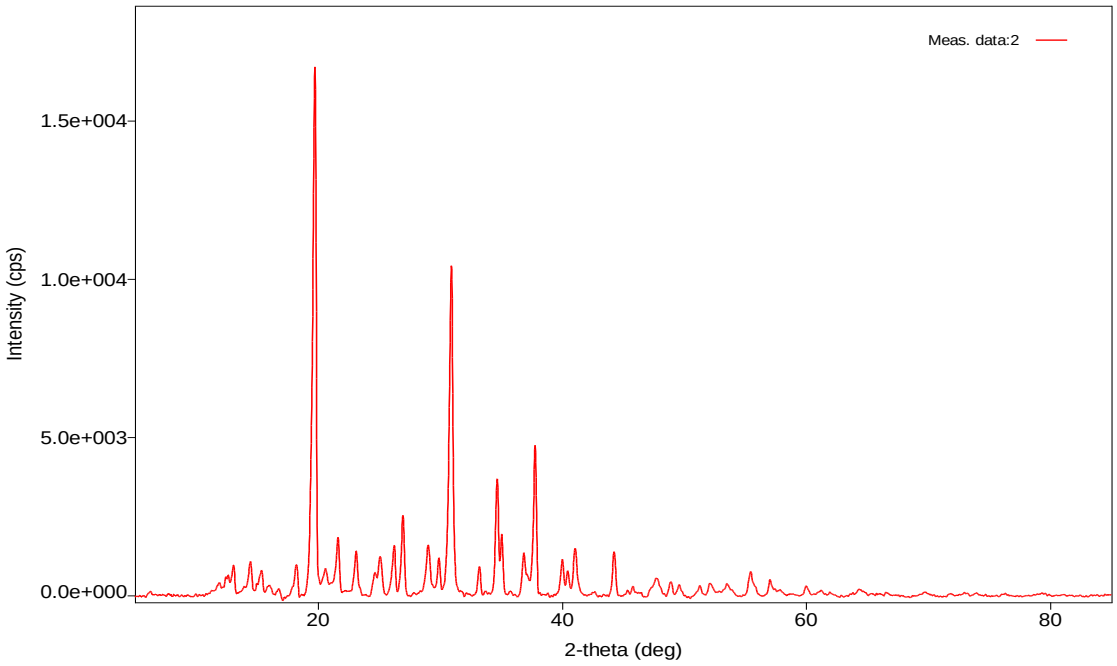


Fig.4. XRD spectra of amla nanoparticles

Anti cancer Activity:(Fig.1,2 and Table 1&2). The objective of this study was to evaluate the cytotoxicity of CeNPs in human bronchoalveolar carcinoma derived cells(A549). This cell line has been widely used in in-vitro cytotoxicity studies[12,13].

The first objective of this study was to evaluate the cytotoxicity of Al_2O_3 nanoparticles (13 nm, 22 nm) in human bronchoalveolar carcinoma-derived cells (A549). This cell line has been widely used in in vitro cytotoxicity studies (Huang, Khor, and Lim 2004; Upadhyay 2003).

On human lung cancer cell line(A 549), S1 and S2 drugs showed to inhibit the growth of cells up to 50% with drug concentration of >80 µg/ml, while the control drug adriamycin inhibited the cell growth up to50% with a drug concentration of < 10 µg/ml.

A description of the characterization of the CeNPs used in this study has been published previously[14].

On human Hepatoma cell line Hep-G2 S1 & S2 drugs showed to inhibit cell growth up to 50% with a drug concentration of < 10 µg/ml.

With the concentration used in the experiment, none of the drug concentrations caused 50 % cell or total inhibition of cell growth.

With the concentrations used in the experiment, the data was non evaluable for 50 % cell kill and total inhibition of cell growth. This could be because of less solubility of the compound.

The percentage of cell growth decreased with increased concentration of drug (>80 µg/ml) with drug S2.But did not show activity as compared to control drug(mild cytotoxicity).

The percentage of cell growth slightly increased with increasing concentration of drug with drug S1 and also failed to show activity (favored growth).

Both the drugs S1 and S2 did not demonstrate activity as GI 50 value >80 µg/ml [to demonstrate activity for extracts GI 50 value should be < 20 µg/ml] and hence are not effective as cytotoxic drug.

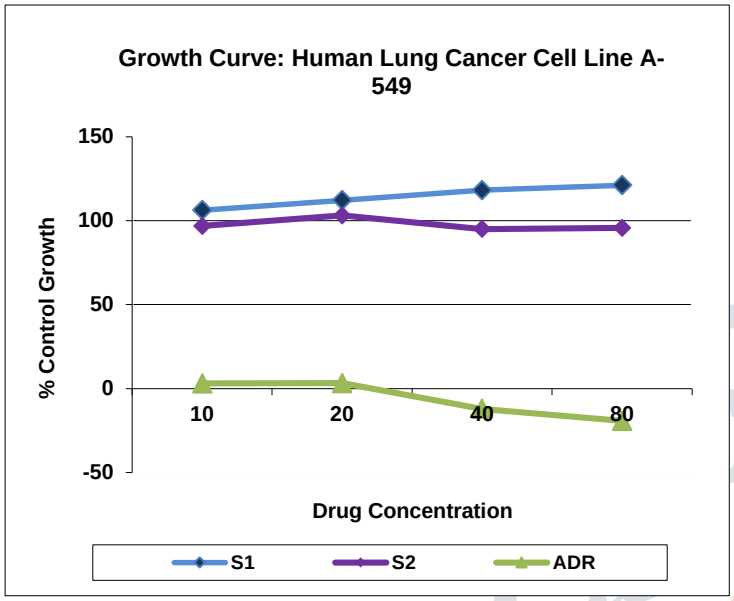


Fig: 1.

	Drug Concentrations (µg/ml) Calculated from graph		
A- 549	**LC50**	**TDI**	**GI50***
S1	**NE**	**NE**	**>80**
S2	**NE**	**NE**	**>80**
ADR	**NE**	**NE**	**<10**

Table. 1

1.00	Human Lung Cancer Cell Line A - 549															
	% control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				**Experiment 2**				**Experiment 3**				**Experiment 4**			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
S1	102.3	111.0	114.2	117.9	109.3	114.0	121.3	125.6	107.1	111.2	118.8	120.0	106.2	112.1	118.1	121.1
S2	100.3	98.9	91.6	92.9	93.6	104.7	93.5	103.6	96.4	105.7	99.8	90.5	103.1	103.1	95.0	95.7
ADR	3.0	3.2	-12.1	-19.3	3.0	3.2	-12.1	-19.3	3.0	3.2	-12.1	-19.3	3.0	3.2	-12.1	-19.3

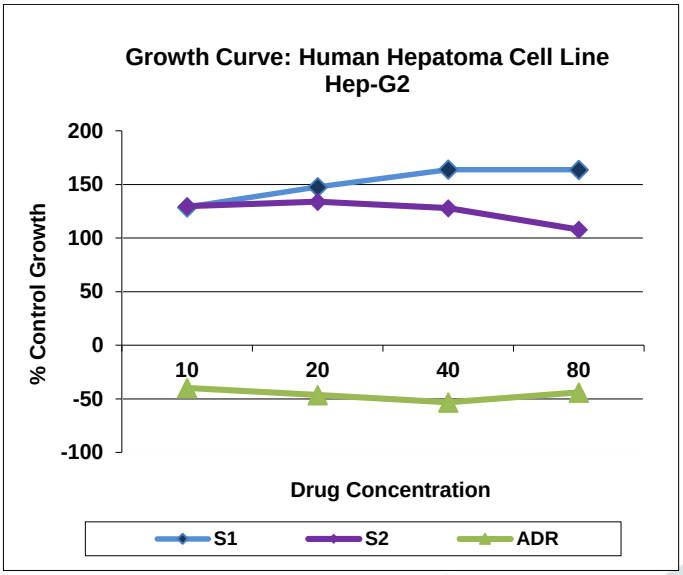
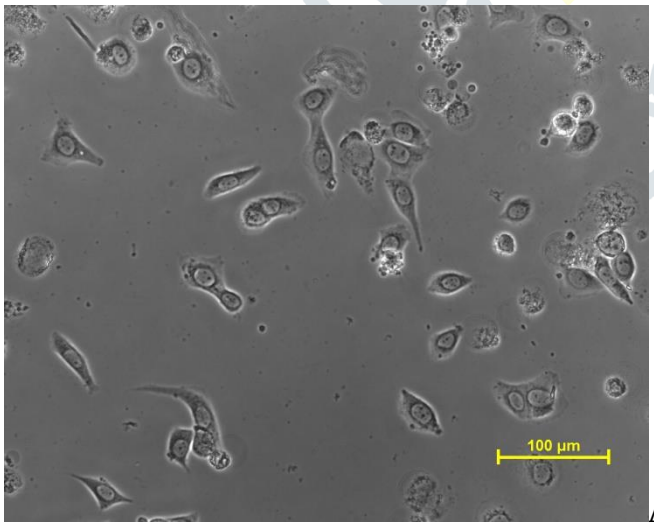


Fig.2

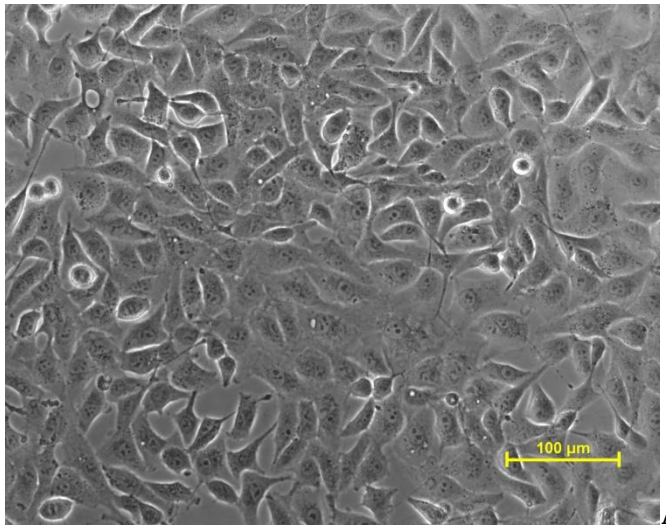
Hep-G2	Drug concentrations (µg/ml) calculated from graph LC50	TGI	GI50*
S1	NE	NE	>80
S2	NE	NE	>80
ADR	NE	NE	<10

Table 2

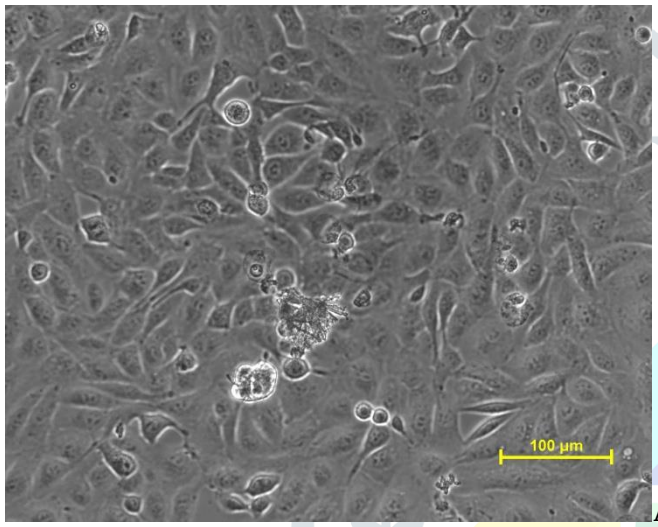
2.00	Human Hepatoma Cell Line Hep-G2															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
S1	134.1	145.2	167.2	166.8	126.5	141.9	157.9	161.1	125.6	155.4	166.6	163.0	128.7	147.5	163.9	163.6
S2	130.8	137.1	137.2	105.2	131.9	119.1	116.4	103.7	126.1	145.4	129.9	114.6	129.6	133.9	127.8	107.8
ADR	-34.5	-47.9	-57.3	-48.3	-41.9	-49.5	-58.8	-52.9	-43.3	-42.1	-43.9	-31.2	-39.9	-46.5	-53.3	-44.1



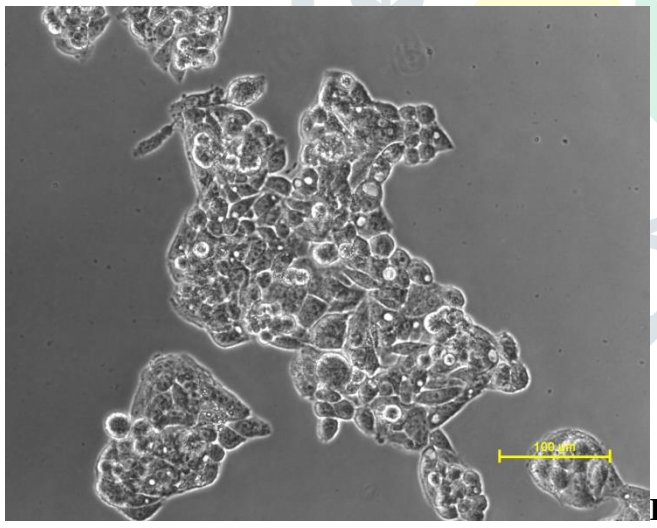
- **A-549 ADR**



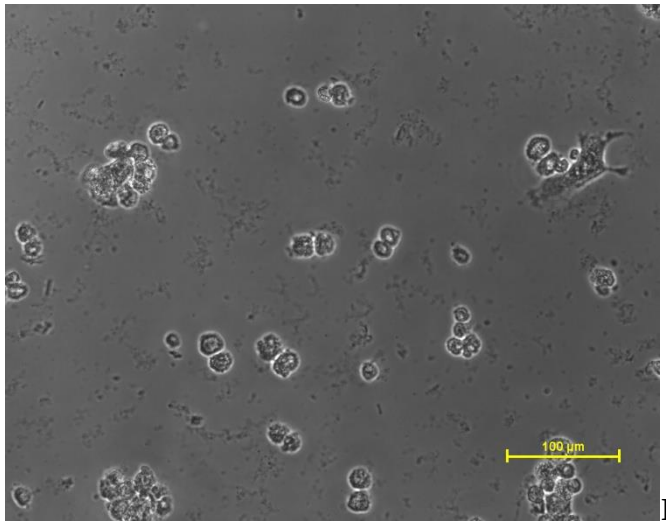
A-549 Control



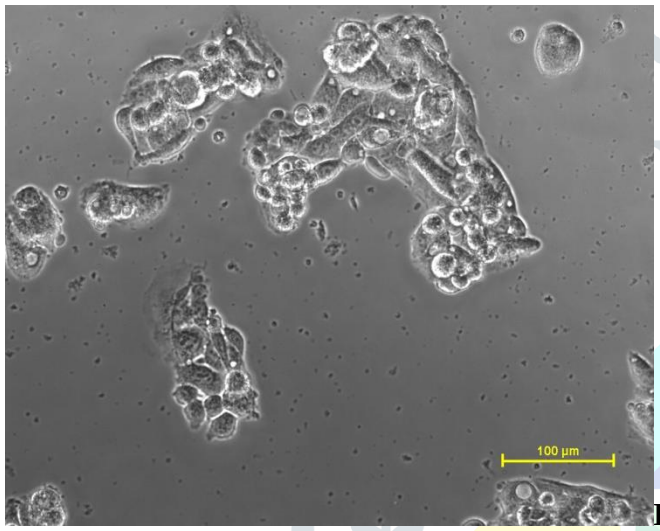
A-549 Fig.1-3



HEP-G2 control



HEP-Positive



HEP-G2 Fig.1-3

Conclusion

The present work indicated the green synthesis of ZrNPs using amla juice extract and use as an anti-cancer agent. The results confirmed that amla uice plays an important role in reduction and stabilization of zirconium. The outcomes of this study illustrate a broad range of applications in medical field.

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